User-Developed Protocol

For purification of SBV RNA from bull semen using the BioSprint® workstations

This protocol has been adapted by customers and is intended as a guideline for the purification of SBV RNA from bull semen using BioSprint workstations and the MagAttract® Virus Mini M48 Kit. **This protocol has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *QIAzol® Lysis Reagent Handbook* and the *MagAttract Virus Mini M48 Kit Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (SDSs), available from the product supplier. QIAzol Lysis Reagent and MagAttract Virus Mini M48 Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user for all protocols

- Pipets and disposable pipet tips with aerosol barriers (20–1000 µl)
- Microcentrifuge tubes (2 ml) for lysis
- Microcentrifuge
- QIAzol Lysis Reagent (cat. no. 79306)
- Chloroform
- Isopropanol
- Ethanol (96–100%)
- MagAttract Virus Mini M48 Kit (cat. no. 955336)
- Vortexer

Equipment and reagents to be supplied by user for the Biosprint96 protocol

- BioSprint 96 workstation
- 96-Well Microplates MP (20) (cat. no. 1031656)
- S-Blocks (24) (cat. no. 19585)
- Large 96 Rod-Cover (16) (cat. no. 1031668)
- "BS96 Viral NA Vet" protocol — QIAGEN Tech Service

Equipment and reagents to be supplied by user for the Biosprint15 protocol

- BioSprint 15 workstation
- BioSprint 15 Plasticware (130) (cat. no.1030058)
- "BS15 Viral mod" protocol — QIAGEN Leipzig R&D

Things to do before starting

- Buffer AW1 is supplied as a concentrate. Add 35 ml ethanol (96–100%) to the bottle containing 27 ml Buffer AW1 concentrate, as described on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 is stable for 1 year when stored at room temperature (15–25ºC).

  Note: Always mix reconstituted Buffer AW1 by shaking before starting a purification procedure.

- Buffer AW2 is supplied as a concentrate. Add 160 ml ethanol (96–100%) to the bottle containing 66 ml Buffer AW2 concentrate, as described on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW2 is stable for 1 year when stored at room temperature (15–25ºC).

  Note: Always mix reconstituted Buffer AW2 by shaking before starting a purification procedure.

- Ensure that MagAttract Suspension B is fully re-suspended. **Vortex for at least 3 minutes** before the first use, and for 1 minute before subsequent uses.

Procedure: Purification of SBV RNA from semen using the BioSprint96

1. **Prepare four S-Blocks and two 96-well microplates according to Table 1. The S-Blocks and microplates are loaded onto the worktable in step 5.**

Table 1. Preparation of S-blocks and microplates

Slot	Message when loading	Plate/block	To add	Volume/well (µl)
6	Load road cover	96-well microplate MP	Large 96-Rod Cover	–
5	Load elution	96-well microplate MP	Buffer AVE	100
4	Load wash 3	S-Block	Ethanol (96-100%)	500
3	Load wash 2	S-Block	Buffer AW2	500
2	Load wash 1	S-Block	Buffer AW1	700
1	Load lysate	S-Block	Lysate*	700

* Added at step 15; volume of lysate includes MagAttract Suspension B and isopropanol.

2. **Switch on the BioSprint 96 at the power switch.**
3. **Slide open the front door of the protective cover.**
4. **Select the protocol "BS96 Viral NA Vet" using the ↓ and ↑ keys. Press "Start" to start the protocol run.**
5. **The LCD displays a message asking you to load slot 6 of the worktable with the 96-rod cover. After loading slot 6, press "Start". The worktable rotates and a new message appears, asking you to load slot 5 with the elution plate. Load slot 5 and**

press “Start” again. Continue this process of pressing “Start” and loading a particular slot until all slots are loaded, except Slot 1. Lysate is added to slot 1 at step 15.

Sample lysis

6. **Pipet 200 µl sample into a 2 ml microcentrifuge tube.**
7. **Add 800 µl of QIAzol Lysis Reagent.**
8. **Homogenize the sample by pipetting the suspension up and down several times. Secure the cap of the tube containing the homogenate and shake vigorously for 15 s.**
9. **Briefly centrifuge the 2 ml tube to remove droplets from the inside of the lid.**
10. **Place the tube containing the homogenate on the benchtop at room temperature for 5 min.**
11. **Add 200 µl of chloroform.**
12. **Secure the cap on the tube containing the homogenate, and shake vigorously for 15 s.**
13. **Place the tube containing the homogenate on the benchtop at room temperature for 10 min.**
14. **Centrifuge at 12,000 x g for 10 min at 4°C.**
15. **Transfer 425 µl of the upper aqueous phase in the S-block.**
    Avoid transferring organic solvents, they inhibit PCR.
16. **Add 20 µl MagAttract Suspension B and 250 µl Isopropanol to the sample.**
17. **Homogenize the sample by pipetting the suspension up and down several times.**
18. **Load the S-block containing the Sample in Slot 1 and press “Start”.**
19. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**
20. **Press “Start” to start sample processing.**
21. **After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press “Start” after removing each plate or block.**
22. **Press “Stop” after all plates and blocks are removed.**
23. **Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**
24. **Switch off the BioSprint 96 at the power switch.**
25. **Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

Procedure: Purification of SBV RNA from semen using the BioSprint15

1. **Switch on the BioSprint 15 at the power switch.**
2. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**
3. **Load up to fifteen 5-tube strips into the tube strip tray. One 5-tube strip is used per sample.**
4. **Add reagents to wells 2-5 of each 5-tube strip according to Table 2. Lysate is added to well 1 at step 15.**

Table 2. Reagents and volumes

Well	Reagent	Volume/well (µl)
1	Lysate*	700
2	Buffer AW1	700
3	Buffer AW2	500
4	Ethanol (96–100%)	500
5	Buffer AVE	100

* Added at step 15; volume of lysate includes MagAttract Suspension B and isopropanol.

Sample lysis

5. **Pipet 200 µl sample into a 2 ml microcentrifuge tube.**
6. **Add 800 µl of QIAzol Lysis Reagent.**
7. **Homogenize the sample by pipetting the suspension up and down several times. Secure the cap of the tube containing the homogenate, and shake vigorously for 15 s.**
8. **Briefly centrifuge the 2 ml tube to remove droplets from the inside of the lid.**
9. **Place the tube containing the homogenate on the benchtop at room temperature for 5 min.**
10. **Add 200 µl chloroform.**
11. **Secure the cap on the tube containing the homogenate, and shake vigorously for 15 s.**
12. **Place the tube containing the homogenate on the benchtop at room temperature for 10 min.**
13. **Centrifuge at 12,000 x g for 10 min at 4°C.**
14. **Transfer 425 µl of the upper, aqueous phase in well 1 of the 5-tube strip.**
    Avoid transferring organic solvents, they inhibit PCR.
15. **Add 20 µl MagAttract Suspension B and 250 µl Isopropanol in well 1 of the 5-tube strip.**
16. **Homogenize the sample by pipetting the suspension up and down several times.**
17. **Slide the tube strip tray fully into the BioSprint 15.**
18. **Close the front door of the BioSprint 15.**
19. **Select the protocol "BS15 Viral mod" using the ↑ and ↓ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**
20. **After the protocol run ends, press "STOP" and slide out the tube strip tray. Transfer the eluted nucleic acids from well 5 of each 5-tube strip to other tubes for long-term storage.**

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